

U.S. Patent Application:

Title: METHODS FOR DIAGNOSING PERVASIVE DEVELOPMENT
DISORDERS, DYSAUTONOMIA AND OTHER NEUROLOGICAL
CONDITIONS

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**METHODS FOR DIAGNOSING PERVASIVE DEVELOPMENT DISORDERS,
DYSAUTONOMIA AND OTHER NEUROLOGICAL CONDITIONS**

CROSS REFERENCE TO RELATED APPLICATION

5 This application is based on, and claims the benefit of, United States Provisional Application No. 60/249,239, filed on November 16, 2000, which is fully incorporated herein by reference.

TECHNICAL FIELD OF THE INVENTION

10 The present invention generally relates to methods for aiding in the diagnosis of disorders including, but not limited to, PDDs (Pervasive Development Disorders), Dysautonomic disorders, Parkinsons disease and SIDS (Sudden Infant Death Syndrome). More particularly, the invention relates to a diagnosis method comprising analyzing a stool sample of an individual for the presence of a biological marker (or marker compound) comprising one or more pathogens, which provides an indication of whether
15 the individual has, or can develop, a disorder including, but not limited to, a PDD, a Dysautonomic disorder, Parkinson's disease or SIDS.

BACKGROUND

 Currently, extensive research is being conducted to determine associations between gastrointestinal dysfunction and a variety of human disorders that, heretofore,
20 have been of unknown etiology. For example, an association between dysautonomic conditions and gastrointestinal dysfunction has been described in U.S. Patent Application Serial No. 09/929,592, filed on August 14, 2001, entitled "Methods For Diagnosing and Treating Dysautonomia and Other Dysautonomic Conditions, which is commonly owned

and fully incorporated herein by reference. Further, a relationship between gastrointestinal conditions and PDDs such as Autism, ADD (Attention Deficit Disorder) and ADHD (Attention Deficit Hyperactivity Disorder) has been described in detail in U.S. Patent Application Serial No. 09/466,559, filed December 17, 1999, entitled
5 “Methods For Treating Pervasive Development Disorders,” and U.S. Serial No. 09/707,395, filed on November 7, 2000, entitled “Methods For Treating Pervasive Development Disorders”, both of which are commonly owned and incorporated herein by reference.

Based on these findings, it is thus desirable to continue research in finding
10 biologic markers of gastrointestinal dysfunction that may aid in the diagnosis of certain diseases and disorders. For example, the effect of various pathogens on the gastrointestinal tract, and the association of such pathogens to disorders such as PDD and dysautonomia, has heretofore not been researched. Various microorganisms that are of interest will now be discussed.

15 *Helicobacter pylori* (*H. pylori*) is generally associated with chronic gastritis and peptic ulcer in children and adults. The prevalence of *H. pylori* is highest in developing countries and lowest in developed countries. Ethnicity, socioeconomic status, household crowding, and other conditions contribute to the formation of *H. pylori* infection. Infection is rarely symptomatic in children, and duodenal ulcers are generally not seen in
20 children less than 10 years of age. Various diseases that are caused, or believed to be caused by *H. pylori* infection are known. For instance, it has been postulated that

H.pylori plays a role in auto-immune athero-sclerosis.

Esophageal reflux disease (GORD) has further been postulated to be caused by H.pylori in a mechanism whereby somatostatin induces the hypothalamus to decrease the release of growth hormone from the pituitary affecting the adrenal control of cortisol. The change in cortisol ultimately affects the gastrin release mechanism causing an increase in acid.

Cryptosporidium parvum can be associated with infections of the gastrointestinal tract in children and in immunocompromised populations. It is generally thought to account for up to 20% of all cases of diarrhea in developing countries and potentially life threatening in children with AIDS due to the induction of severe malnutrition. These infections are generally asymptomatic and occur in tandem with other infections such as one with Giardia.

In 1993, a large outbreak of Cryptosporidium parvum occurred in Milwaukee Wisconsin in which 400,000 people were affected. It has a seasonal effect of being more prevalent in the late summer in children under the age of 15 years.

Giardia lamblia is a common cause of diarrhea in humans and other mammals throughout the world. In its most severe form, it has been found to cause infectious lymphocytosis. Although rare, infection with Giardia can be protracted and debilitating. Giardia lamblia is a flagellate that encysts, and generally does not cause symptomatology. However, when found in the trophozoite form, severe diarrhea can result. Symptoms can include diarrhea, vomiting, fatigue, and growth retardation in

children. Malabsorption results from infection with the trophozoite form, and potential blockage of the microvilli of the intestines occurs. There may be an interaction between decreased levels of IgA in the gastrointestinal system and giardiasis.

Clostridium infections of the gastrointestinal tract are of the *perfringes*, *botulinum* and *difficile* varieties. *Perfringens* food poisoning is the term used to describe the common foodborne illness caused by *C. perfringens*. A more serious but rare illness is also caused by ingesting food contaminated with Type C strains. The latter illness is known as enteritis necroticans. The common form of *perfringens* poisoning is characterized by intense abdominal cramps and diarrhea which begin 8-22 hours after consumption of foods containing large numbers of those *C. perfringens* bacteria capable of producing the food poisoning toxin. The illness is usually over within 24 hours but less severe symptoms may persist in some individuals for 1 or 2 weeks. A few deaths have been reported as a result of dehydration and other complications. Necrotic enteritis caused by *C. perfringens* is often fatal. This disease also begins as a result of ingesting large numbers of the causative bacteria in contaminated foods. This disease is a food infection; only one episode has ever implied the possibility of intoxication (i.e., disease from preformed toxin).

Clostridium difficile is an infection generally caused by changes in the intestinal mucosa. Those changes are caused by an overuse of antibiotics creating an intestinal environment favorable to the infiltration with *Clostridium difficile*. Infection with *C. difficile* is generally debilitating and *C. difficile* is a gram-positive, spore forming,

anaerobic bacillus which can produce toxin-mediated diarrhea or pseudomembranous colitis. It has been isolated from soil, sand, hay, and animal dung. *C. difficile* colonization of the colon occurs in 2% - 3% of healthy adults. Following exposure to antibacterial agents, the rate of asymptomatic colonization in adults averages between 5% to 15%, but
5 rates as high as 46% have been reported. Carriage rates of up to 70% have been reported in children below the age of one year, but by two years of age the "normal" colonic flora is established and the frequency of colonization decreases to that of healthy adults. Of interest is that healthy children less than one year of age are the only population in which *C. difficile* toxins are frequently detected in the stool in the absence of clinical symptoms.
10 One suggestion advanced to explain this observation is that the infant's gut cannot respond to the toxin.

Clostridium botulinum is an anaerobic, spore-forming rod that produces a potent neurotoxin. The spores are heat-resistant and can survive in foods that are incorrectly or minimally processed. Seven types (A, B, C, D, E, F and G) of botulism are recognized,
15 based on the antigenic specificity of the toxin produced by each strain. Types A, B, E and F cause human botulism. Types C and D cause most cases of botulism in animals. Animals most commonly affected are wild fowl and poultry, cattle, horses and some species of fish. Although type G has been isolated from soil in Argentina, no outbreaks involving it have been recognized. Foodborne botulism (as distinct from wound botulism
20 and infant botulism) is a severe type of food poisoning caused by the ingestion of foods containing the potent neurotoxin formed during growth of the organism. The toxin is heat

labile and can be destroyed if heated at 80oC for 10 minutes or longer. The incidence of the disease is low, but the disease is of considerable concern because of its high mortality rate if not treated immediately and properly. Most of the 10 to 30 outbreaks that are reported annually in the United States are associated with inadequately processed, home-
5 canned foods, but occasionally commercially produced foods have been involved in outbreaks. Sausages, meat products, canned vegetables and seafood products have been the most frequent vehicles for human botulism.

The life cycle of *Entamoeba histolytica* involves trophozoites (the feeding stage of the parasite) that live in the host's large intestine and cysts that are passed in the host's
10 feces. Humans are infected by ingesting cysts, most often via food or water contaminated with human fecal material. The trophozoites can destroy the tissues that line the host's large intestine, so of the amoebae infecting the human gastrointestinal tract, *E. histolytica* is potentially the most pathogenic. In most infected humans the symptoms of "amoebiasis" (or "amebiasis") are intermittent and mild (various gastrointestinal upsets,
15 including colitis and diarrhea). In more severe cases the gastrointestinal tract hemorrhages, resulting in dysentery. In some cases the trophozoites will enter the circulatory system and infect other organs, most often the liver (hepatic amoebiasis), or they may penetrate the gastrointestinal tract resulting in acute peritonitis; such cases are often fatal. As with most of the amoebae, infections of *E. histolytica* are often diagnosed
20 by demonstrating cysts or trophozoites in a stool sample. Infections that sometimes last for years may be accompanied by no symptoms, vague gastrointestinal distress, and/or

dysentery (with blood and mucus). Most infections occur in the digestive tract but other tissues may be invaded. Complications include ulcerative and abscess pain and, rarely, intestinal blockage. Onset time is highly variable. It is theorized that the absence of symptoms or their intensity varies with such factors as strain of amoeba, immune health of the host, and associated bacteria and, perhaps, viruses. The amoeba's enzymes help it to penetrate and digest human tissues; it secretes toxic substances.

No extensive research is known to have been conducted heretofore to determine correlations and associations regarding the presence of pathogens in the gastrointestinal tract of individuals in, e.g., PDD, Parkinson's and Dysautonomia populations. Based on the findings described herein in accordance with the present invention, correlations and associations are found to exist between various disorders such as Autism, Parkinson's, ADD, ADHD and Dysautonomia, for example, and the presence of pathogens in an individual's digestive tract .

SUMMARY OF THE INVENTION

The present invention generally relates to methods for aiding in the diagnosis of disorders including, but not limited to, PDDs, Dysautonomic disorders, Parkinson's disease and SIDS. More particularly, the invention relates to a diagnosis method comprising analyzing a stool sample of an individual for the presence of a biological marker comprising one or more pathogens, which provides an indication of whether the individual has, or can develop, a disorder including, but not limited to, a PDD, Dysautonomia, Parkinson's disease and SIDS. In a preferred embodiment, a stool

immunoassay is used to determine the presence of antigens in a stool sample, wherein such antigens are associated with one or more pathogens including, but not limited to, Giardia, Cryptosporidium, E. histolytica, C. difficile, Adenovirus, Rotavirus or H.pylori.

In another aspect of the invention, pathogens including, but not limited to, Giardia, Cryptosporidium, E. histolytica, C. difficile, Adenovirus, Rotavirus, and H pylori, comprise biological markers whose presence in a stool sample, for example, are efficacious for determining whether an individual, especially a child, has, or can potentially develop, a disorder including, but not limited to, a PDD, Dysautonomia, Parkinsons disease, SID, and/or other neurological disorders.

These and other aspects, features, and advantages of the present invention will be described and become apparent from the following detailed description of preferred embodiments, which is to be read with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a table diagram illustrating various pathogens that tested positive in stool samples of individuals having a Dysautonomic disorder;

FIG. 2 is a table diagram illustrating various pathogens that tested positive in stool samples of individuals having Parkinson's, as compared with stool results of individuals not having Parkinson's disease;

FIG. 3 is a table diagram illustrating various pathogens that tested positive in stool samples of individuals having ADD or ADHD, as compared with stool results of

individuals not having ADD or ADHD; and

FIG. 4 is a table diagram illustrating various pathogens that tested positive in stool samples of individuals having a PDD.

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DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is directed to methods for aiding in the diagnosis of various human disorders, such as PDD, Dysautonomia, Parkinson's, SIDS, etc. In one aspect, a method comprises analyzing stool samples of an individual to determine the presence of pathogens including, but not limited to, Giardia, Cryptosporidium, Entamoeba histolytica, Adenovirus, Rotavirus, H pylori, Cyclospora, Microsporidia, and/or Isospora belli.

Preferably, the presence of one or more pathogens is determined using a stool immunoassay to determine the presence of antigens in a stool sample, wherein such antigens are associated with one or more pathogens including, but not limited to, Giardia, Cryptosporidium, E. histolytica, C. difficile, Adenovirus, Rotavirus or H.pylori.

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In particular, a stool immunoassay results in the determination of the presence of a particular antigen (usually a protein) that the particular pathogen leaves behind (i.e., each microorganism is associated with a specific antigen). This antigen represents a pathogen, and is recognized by the gastrointestinal tract of the individual as a foreign protein. In accordance with one aspect of the invention, the presence of one or more antigens,

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regardless of the quantitative level, comprises a biological marker for determining if the

person, especially a child, may either have or develop a disorder such as a PDD,
Dysautonomia, Parkinson's, or SIDs.

Until now, there has been no known methods for analyzing stool samples to
determine the presence of pathogens as biological markers to allow early diagnosis or
5 screening of such disorders or conditions. It is postulated, for example, that the presence
of antigen(s) and/or the microscopic presence of such organisms may signal the formation
of a dysbiosis, and ultimately the formation of a malabsorption syndrome. This
malabsorption syndrome can predispose the individual to the formation of a disorder such
as autism, ADD, ADHD, SIDS, PDD, tourettes, OCD and other neurological conditions.
10 In particular, the formation of malabsorption syndrome can affect, for instance, proper
and essential protein digestion/absorption. And in the absence of proper protein
digestion/absorption, the amino acids necessary for, e.g., the growth and development or
normal functioning of certain chemical processes of individuals are absent.

Consequently, it is postulated, for example, that a lack of proper protein
15 absorption, for instance, of an individual, especially children, can lead to various
disorders such as autism, other PDDs, SIDS, and other disorders mentioned herein.
Indeed, as described in each of the above-incorporated U.S. Patent Applications,
abnormal protein digestion is found to occur in the PDD and dysautonomic populations.
For instance, it was further determined that a sub-population of individuals suffering from
20 ADD, ADHD and autism as well as a sub-population of those with dysautonomic
conditions had an abnormal level of the enzyme chymotrypsin, indicated pancreatic

insufficiency as a component of such disorders.

The following case studies indicate that there are correlations between the development of various disorders and the presence of microorganisms in an individual's digestive tract. It is to be understood that these examples are set forth by way of illustration only, and nothing therein shall be taken as a limitation upon the overall scope of the invention.

Case 1:

Stool sample were collected from two children diagnosed as having Familial Dysautonomia. The stool samples were analyzed for the presence of pathogens. As shown by the table in Fig. 1, the stool sample of Child 1 tested positive for H. pylori, Giardia, and Cryptosporidium. Further, the stool sample of Child 2 tested positive for H. pylori, Giardia, Cryptosporidium and Rotavirus.

Case 2:

Stool samples were collected from 15 individuals diagnosed as having Parkinson's disease. The stool samples were analyzed for the presence of pathogens. Further, stool samples were collected from an additional 15 individuals who were not diagnosed as having Parkinson's disease, nor having known familial association with Parkinson's or known GI conditions. These stool samples were also analyzed for the presence of pathogens.

The table in Fig. 2 illustrates the result of this study. As shown, the stools of each of the 15 individuals diagnosed as having Parkinson's disease tested positive for various pathogens including *H. pylori*, *Cryptosporidium*, *E. hystolytica*, *Giardia*, *Rotavirus*, *Camphylobacter*, and/or *C difficile*. On the other hand, virtually all the stools of each of the 15 individuals not diagnosed as having Parkinson's disease tested negative for such pathogens.

Case 3:

Stool samples were collected from 13 children diagnosed as having either ADD or ADHD and analyzed for the presence of pathogens. Further, stool samples were collected from an additional 14 children not diagnosed as having ADD or ADHD and analyzed for the presence of pathogens.

The table in Fig. 3 illustrates the result of this study. As shown, the stools of each of the 13 children diagnosed as having either ADD or ADHD tested positive for various pathogens including as *H. pylori*, *Cryptosporidium*, *E. hystolytica*, *Giardia*, *Rotavirus*, *Camphylobacter*, and/or *C difficile*. On the other hand, virtually all the stools of each of the 14 children not diagnosed as having ADD or ADHD tested negative for such pathogens.

Case 4:

Stool samples were collected from 19 children diagnosed as having Autism (via a

CARS or ADOS test) and analyzed for the presence of pathogens. The results of this study are shown in Fig. 4. As shown, the stools of each of the 19 children diagnosed as having Autism tested positive for various pathogens including as *H. pylori*, *Cryptosporidium*, *E. histolytica*, *Giardia*, *Rotavirus*, *Camphylobacter*, and/or *C difficile*.

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The results of these case studies indicate that there are correlations between the development of various disorders (such as Autism, Parkinson's, ADD and ADHD) and the presence of pathogens and/or corresponding antigens in an individual's digestive tract. It is postulated that these pathogens and/or corresponding antigens either promote gastrointestinal dysfunction or have some other direct or indirect effect on the individual, thereby causing such disorders. Further, it is possible that certain mechanisms associated with such disorders can be the cause of a proliferation of one or more pathogens in the gastrointestinal tract of an individual. Again, it is to be understood that nothing therein shall be taken as a limitation upon the overall scope of the invention.

For instance, although Case Study 4 involves Autism, based on the correlations described herein, it is believed that the present invention may be implemented for aiding in the diagnosis of other various PDDs such as Aspergers syndrome and other related disorders. Furthermore, although Case Study 1 involves Familial Dysautonomia, based on the correlations described herein, it is believed that the present invention may be implemented for aiding in the diagnosis of various dysautonomic disorders and dysautonomic conditions, including, but not limited to, Familial Dysautonomia (or Riley-

Day Syndrome), Guillaine-Barre Syndrome (GBS) (acute idiopathic polyneuropathy), fetal fatal insomnia (FFI), diabetic cardiovascular neuropathy, Hereditary Sensory and autonomic neuropathy type III (HSAN III), central autonomic disorders including multiple system atrophy (Shy-Drager syndrome), orthostatic intolerance syndrome

5 including mitral value prolapse, postural tachycardia syndrome (POTS), and idiopathic hypovolemia, dysautonomic syndromes and disorders of the catecholamine family including baroreflex failure, dopamine-β-Hydroxylase deficiency, pheochromocytoma, chemodectoma, familial paraganglioma syndrome, tetrahydrobiopterin deficiency, aromatic-L-amino acid decarboxylase deficiency, Menke's disease, monoamine oxidase

10 deficiency states, and other disorders of dopamine metabolism, dysautonomic syndromes and disorders of the cardiovascular system, Chaga's disease, Diabetic autonomic failure, pure autonomic failure, syncope, hypertension, cardiovascular disease, renal disease and SIDS. Further, the present invention is believed to be efficacious for diagnosing other neurological disorders such as OCD (obsessive compulsive disorder) and Tourette's

15 syndrome.

In summary, a method according to the present invention for aiding in the diagnosis of a disorder comprises analyzing stool samples of an individual to determine the presence of one or more pathogens including, but not limited to, *H. pylori*, *Cryptosporidium*, *Entamoeba histolytica*, *Giardia*, *Rotavirus*, *Camphylobacter*, and/or *C. difficile*. Other pathogens that may be analyzed include, for example, *Adenovirus*,

20 *Cyclospora*, *Microsporidia*, and/or *isospora belli*. In a preferred embodiment, the presence

of one or more pathogens is determined by a stool immunoassay to determine the presence of associated antigens. The presence of one or more pathogens comprises a biological marker for determining if an individual, especially a child, may either have or develop a disorder, including, but not limited to, PDD (such as Autism), Dysautonomia
5 (or other dysautonomic conditions), Parkinson's disease, SIDS, or other dysautonomic and/or neurological disorders.

Although illustrative embodiments have been described herein with reference to the accompanying drawings, it is to be understood that the present invention is not limited to those precise embodiments, and that various other changes and modifications may be
10 effected therein by one skilled in the art without departing from the scope or spirit of the invention. All such changes and modifications are intended to be included within the scope of the invention as defined by the appended claims.